NAG9-1155: Nutritional Intervention to Improve Muscle Protein Metabolism During Stress

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Introduction

The Critical Path Roadmap Mission Statement for muscle alterations and atrophy during spaceflight states:

> *"Evidence demonstrates that living and working in the weightlessness induces serious, non-permanent changes in muscles which may inhibit piloting, egress, and useful work. Reduced workload causes muscles to decrease in size, strength and endurance. There are changes also in muscle protein composition. Mitigation requires: understanding of hormonal and nutritional aspects of muscle change in weightlessness; development of prescription modalities and identification of compliance factors; development and testing of pharmacological countermeasures and effective exercise regimes; and an understanding of how skeletal muscle deficits are reflected in other organ systems. Technology developments include: advanced noninvasive monitoring of hormonal levels, muscle function and strength, and nutritional status; individual exercise regimes; and extended shelf-life pharmacological agents."*

This report will demonstrate how research, supported by NAG-1155 and performed in our laboratory specifically addresses several core directives of the mission statement. Specifically, research supported by NAG-1155 funding:

- Furthered our understanding of hormonal and nutritional aspects of muscle change in weightlessness.
- Provided important preliminary information that is being utilized for development of nutritional and exercise prescription modalities.
- Validated the use of effective nutritional countermeasures and provided important preliminary information for further research using a combination of nutrition, exercise and pharmacological intervention regimes.

Background:

Debilitating injury or trauma is associated with muscle wasting and a progressive loss of functional capacity. This imbalance between muscle protein synthesis and breakdown is likely facilitated by an increased circulating level of the stress hormone cortisol [1, 2]. The hypercortisolemic response is generally proportional to the severity of an injury and has been shown to increase skeletal muscle proteolysis, particularly when combined with immobilization or bedrest [2-4]. The metabolic changes that result in a loss of muscle tissue following injury can be broadly characterized as occurring as a result of two main physiological responses: i) changes in postabsorptive protein metabolism, ii) alteration in the anabolic response to feeding. Several studies have characterized changes in postabsorptive protein metabolism in response to hypercortisolemia [1, 4-6]. In general, these studies demonstrate that in the postabsorptive state, cortisol increases whole body proteolysis [6], increases plasma insulin levels [5, 6] and raises plasma amino acid concentrations [1, 4-6].

While physical interventions such as exercise may provide a potent anabolic stimulus, exercise may not be a feasible countermeasure in all situations, particularly those involving injury or trauma. Consequently, there is need to examine additional strategies, such as nutrition, that may arrest the cachetic process. Following severe injury or traumatic insult, the normal anabolic stimulus to feeding is disrupted. Even with tremendously elevated caloric intakes, some severely injured individuals fail to maintain lean body mass [7, 8]. Further, while chronic outcome measurements such as body-weight, strength and functional ability clearly demonstrate that protein catabolism exceeds muscle protein synthesis following injury, it is not clear if this disruption of physiological homeostasis can be blunted or offset by a nutritional intervention.

We have recently shown in healthy subjects that ingestion or infusion of essential amino acids (EAA) provides a potent anabolic stimulus [9, 10]. Further, preliminary data from our laboratory suggests that the anabolic response to EAA ingestion is substantially greater than a mixed meal or even an intact protein source.

Project Aims

The purpose of this study was to determine if the net muscle catabolism that accompanies hypercortisolemia could be ameliorated with a bolus ingestion of 15g of EAA. To compliment the protein kinetics data, we chose two independent, primary endpoint measurements, muscle fractional synthetic rate (FSR) and net phenylalanine balance. We hypothesized that EAA ingestion would be effective in stimulating muscle protein anabolism during acute hypercortisolemia.

Methods:

Twelve healthy male and female subjects (mean±SEM: 28±5yrs, 165±6cm, 70±2kg) were randomly assigned to one of two experimental conditions. In the amino acid group (AA; 2 male, 4 female), we used established stable isotope methodology [11, 12] to measure muscle protein kinetics before and after a bolus ingestion of 15g EAA. In the second group (C+AA; 2 male, 4 female) we induced hypercortisolemia and then measured muscle protein kinetics before and after ingestion of 15g EAA.

Experimental Protocols

This study was approved by the Institutional Review Board at The University of Texas Medical Branch (UTMB). After explaining the experimental protocol and risks of the study, written informed consent was obtained from each volunteer. The study time-line is presented in Figure 1.

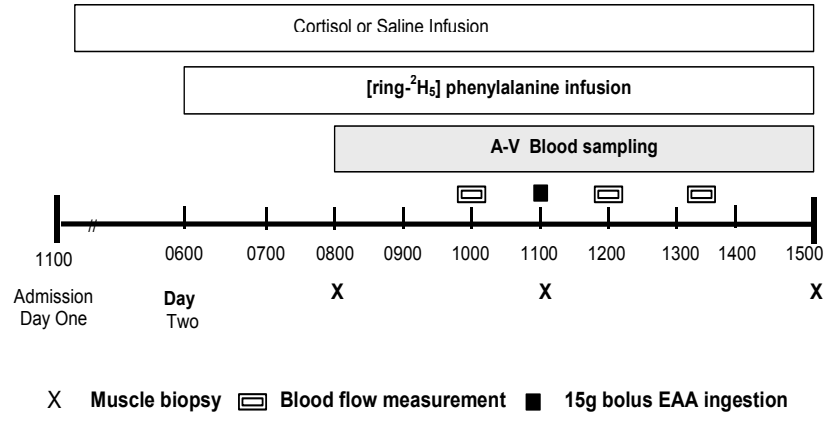

Figure 1. Study time line and infusion protocol

At 1100 hrs on Day One of the protocol, subjects were admitted to the General Clinical Research Center (GCRC) at UTMB, given a standard meal and then allowed only water until the completion of the study. At 1200 hrs an 18-gauge, polyethylene catheter was inserted into a forearm vein for infusion of hydrocortisone sodium succinate (Infusion rate: $80\mu g \cdot kg^{-1} \cdot hr^{-1}$), (Solu-Cortef, Upjohn Company, MI) or placebo (0.9% saline). The following morning at 0600 hrs, a second 18-gauge, polyethylene catheter was inserted into a peripheral forearm vein. After obtaining baseline blood samples, a primed-($2\mu mol \cdot kg^{-1}$) continuous infusion ($0.05\mu mol \cdot kg^{-1} \cdot min^{-1}$) of [ring-$^2H_5$] phenylalanine ($d_5$-Phe) was started. At 0700, polyethylene catheters (3 Fr, 8 cm) were inserted into the femoral vein and femoral artery of one leg under local anesthesia. Arterial and venous blood samples were obtained at 10-20 minute intervals before and after the ingestion of the EAA drink for determination of amino acid kinetics and plasma concentrations of glucose, insulin and cortisol. The femoral artery catheter was also used for indocyanine green (ICG) infusion ($IR=0.5\ mg \cdot min^{-1}$) for the spectrophotometric (λ =805nm) determination of leg blood flow [13]. Urine samples were collected over a 24hr period from the start of the cortisol/saline infusion (1200 Day one) for analysis of 24hr urinary cortisol levels. Muscle biopsies (50 mg) were taken from the lateral portion of the vastus lateralis approximately, 10-15 cm above the knee using a 5mm Bergstrom biopsy needle [14].

The composition of the EAA drink approximated the distribution of essential amino acids in skeletal muscle (Table 1).

Table 1. The distribution of essential amino acids in the 15g EAA drink.

Amino Acid	Grams	% of Total Amino Acids
Histidine	1.64	10.9
Isoleucine	1.56	10.4
Leucine	2.79	18.6
Lysine	2.33	15.5
Methionine	0.46	3.1
Phenylalanine	2.33	15.5
Threonine	2.20	14.7
Valine	1.73	11.5
Total	**15**	**100**

An additional 0.186g of [ring-$^2H_5$] phenylalanine was added to the EAA drink to maintain blood isotopic enrichment. The amino acids were dissolved in 250ml of a non-caloric, non-caffeinated soft-drink and consumed as a bolus at 1100.

Results:

Plasma and urinary cortisol

Plasma cortisol levels were significantly elevated in the C+AA group ($p<0.05$) (Fig. 2). 24hr urinary cortisol levels were 67±15 and 773±136 μg·24hr^{-1} for the AA and C+AA groups respectively ($P<0.05$).

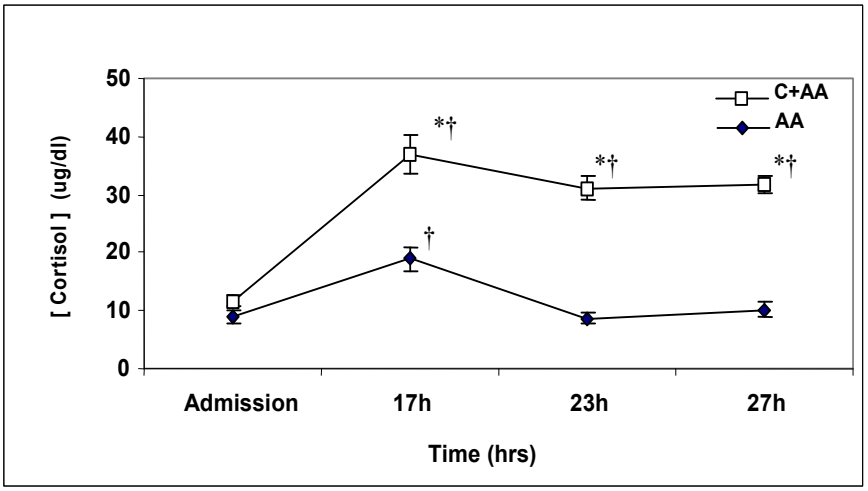

Figure 2. Plasma cortisol levels. * denotes significant difference from control (AA) group: $p<0.05$. † denotes significant difference from baseline (pre): $p<0.05$.

Phenylalanine enrichment and concentration

Arterial and venous phenylalanine concentrations are presented in Figure 3. Postabsorptive kinetic values are presented in Table 2.

Table 2. Postabsorptive postabsorptive phenylalanine kinetics across the leg with (C+AA) and without (AA) hypercortisolemia.

Postabsorptive Variable	**Saline (AA)**	**Cortisol (C+AA)**	**Pvalue**
1. Plasma cortisol: (μg/dl) (<0.001)	9.0±1.0	36.5±2.1	
2. Plasma insulin: (μIU/ml) (<0.001)	4.7±1.0	15.3±1.1	
3. Blood glucose: (mg/dl) (<0.001)	82.0±2.4	109.5±2.6	
4. Arterial Concn: (nmol Phe/ml)	58.8±3.4	72.5±4.1	(0.001)
5. Venous Concn: (nmol Phe/ml)	64.1±3.8	78.3±4.9	(0.002)
6. Muscle IC Concn: (nmol Phe/ml)	70.8±6.7	92.5±5.2	(0.001)
7. Arterial Enrichment: (%)	7.4±0.4	7.1±0.2	ns
8. Venous Enrichment: (%)	6.1±0.5	6.0±0.2	ns
9. Delivery (F_{in}): (nmol Phe/min/100ml leg)	174.7±18.1	274.3±24.5	(0.005)
10. Release (F_{out}): (nmol Phe/min/100ml leg)	191.6±19.3	298.0±26.5	(0.005)
11. Fractional synthetic rate: (%/hr)	0.083±0.001	0.073±0.007	ns
12. Rate of appearance: (nmol Phe/min/100ml leg)	36.3±5.7	47.9±5.3	ns
13. Rate of disappearance: (nmol Phe/min/100ml leg)	19.3±5.5	24.2±3.6	ns
14. Net balance: (nmol Phe/min/100ml leg)	-15.6±3.2	-22.6±7.5	ns

Values are means±SEM from the postabsorptive post-absorptive period.

Following ingestion of 15g EAA, arterial and venous phenylalanine concentrations in the AA and C+AA groups increased significantly ($P<0.05$). However, the magnitude of the arterial and venous concentration change was significantly blunted by cortisol infusion {arterial peak values: 272.4±12.1 (AA) vs. 208.1±17.8 nmol Phe·ml^{-1} (C+AA)}($P<0.05$). Further, hypercortisolemia resulted in arterial phenylalanine concentrations remaining elevated for a longer period of time post-EAA drink (Fig. 3).

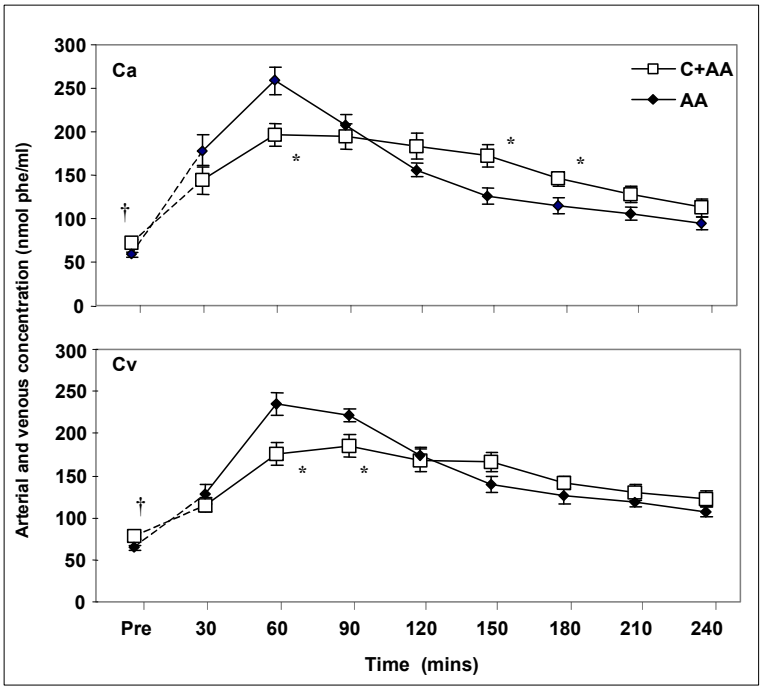

Figure 3. Femoral artery and vein phenylalanine concentrations before (Pre) and following ingestion of 15g EAA. * denotes significant difference from control (AA) group: $p<0.05$. † denotes that all post-EAA values were different from baseline (pre): $p<0.05$.

During the post-absorptive period, hypercortisolemia increased muscle intracellular phenylalanine concentrations (Table 1). EAA ingestion further increased muscle intracellular phenylalanine concentrations, with the greatest increase occurring in the C+AA group (Fig. 4).

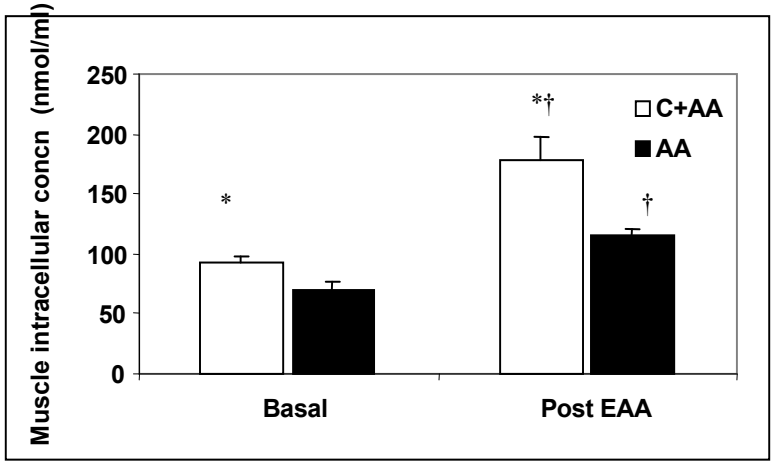

Figure 4. Muscle intracellular phenylalanine concentrations from biopsy samples obtained during the postabsorptive period and 4hrs following EAA ingestion. * denotes significant difference from control (AA) group: $p<0.05$. † denotes significant difference from the postabsorptive period: $p<0.05$.

Rate of appearance (R_a) and disappearance (R_d)

During the postabsorptive period, phenylalanine rate of appearance R_a, a reflection of leg muscle protein breakdown, was not significantly increased by acute hypercortisolemia (Table 1).

Hypercortisolemia did not alter phenylalanine rate of disappearance (R_d), a reflection of muscle protein synthesis, during the postabsorptive period. However, following EAA ingestion, R_d was blunted by hypercortisolemia, yet remained above postabsorptive levels for a greater period of time ($P<0.05$; Fig. 5).

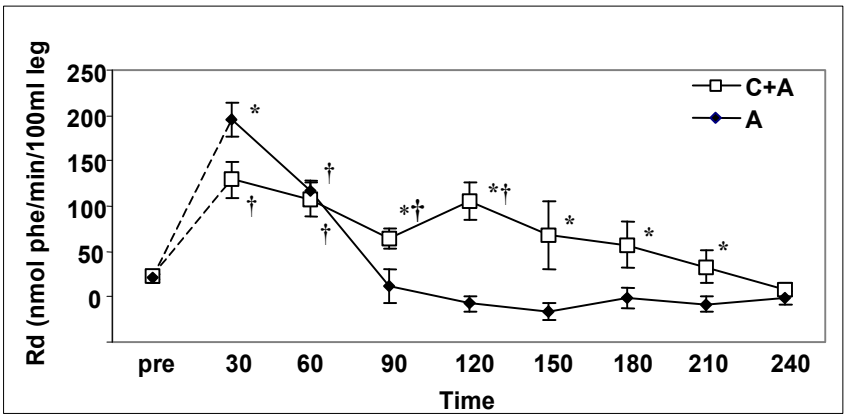

Figure 5. Rate of phenylalanine disappearance (R_d) before (Pre) and following ingestion of 15g EAA. * denotes significant difference from control (AA) group: $p<0.05$.† denotes significant difference from baseline (pre): $p<0.05$.

Net balance

No differences in net phenylalanine balance (NB) were identified while subjects remained in the post-absorptive state. Both groups experienced a significant increase in net phenylalanine balance following ingestion of 15g EAA, however the response in

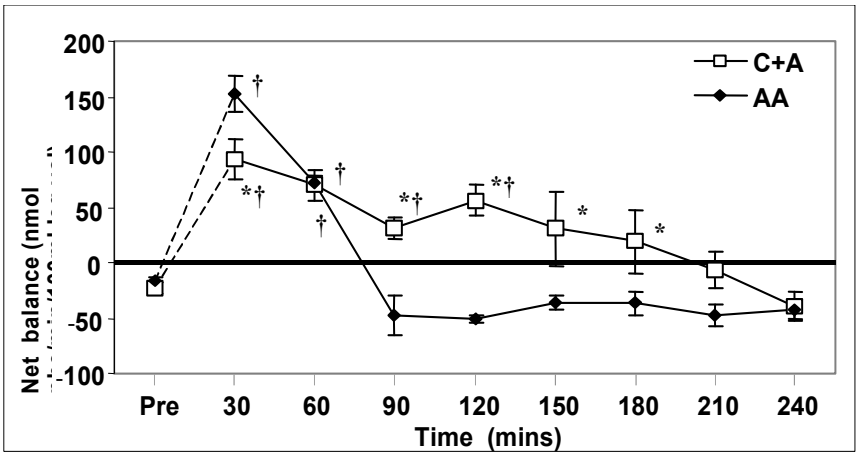

Figure 6. Phenylalanine net balance across the leg before (Pre) and following ingestion of 15g EAA. * denotes significant difference from control (AA) group: $p<0.05$. † denotes significant difference from baseline (pre): $p<0.05$.

the C+AA group was blunted but remained positive for a greater period of time (Fig. 6).

Calculation of net phenylalanine uptake (NB area under the curve: above baseline NB=0) by the leg for the immediate 1hr and 3hr post-drink periods also reflect the blunted, yet prolonged period of positive net balance associated with hypercortisolemia. (Fig. 7).

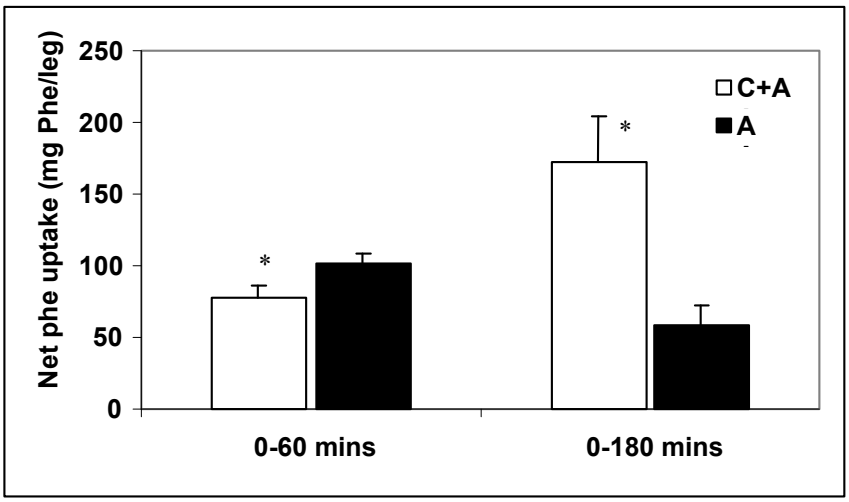

Figure 7. Net phenylalanine uptake by the leg from 0-60 mins and 0-180 mins following 15g EAA ingestion. * denotes significant difference from control (AA) group: $p<0.05$.

Muscle fractional synthetic rate (FSR)

Both groups experienced an increase in mixed muscle FSR in response to ingestion of 15g EAA ($P<0.05$). However, no between group differences were evident during the pre- or post-drink periods, or in absolute changes in FSR following EAA ingestion.

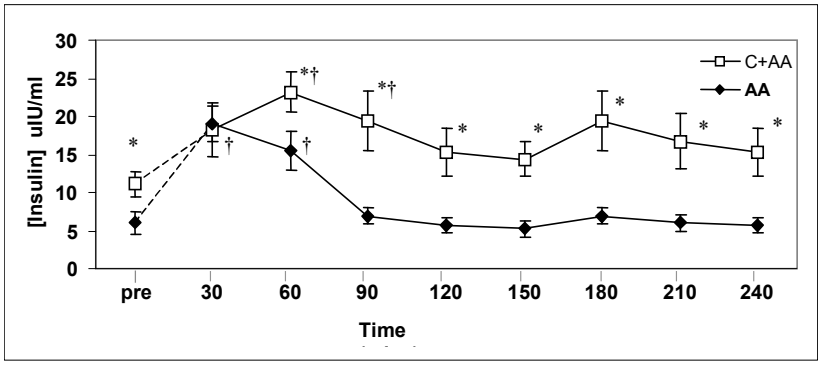

Figure 8. Plasma insulin concentrations before (Pre) and following ingestion of 15g EAA. * denotes significant difference from control (AA) group: $p<0.05$. † denotes significant difference from baseline (pre): $p<0.05$.

Insulin and glucose

Postabsorptive plasma insulin concentrations were elevated in response to hypercortisolemia. Following EAA ingestion, insulin levels increased in both

groups (P<0.05), but remained significantly higher in the C+AA group (P<0.05), (Fig. 8). Hypercortisolemia also increased plasma glucose concentrations (p<0.05).

Discussion:

Following severe injury, individuals continue to lose muscle mass despite elevated caloric intake [7, 8]. This loss of lean body mass, while partly due to reduced physical activity, is likely facilitated by a concomitant increase in plasma cortisol levels [1, 2]. Normal circulating levels of cortisol follow a diurnal pattern but are typically in the range of 5-15 $\mu g \cdot dl^{-1}$. The goal of this study was to induce acute hypercortisolemia ($\sim 35 \mu g \cdot dl^{-1}$) to mimic levels observed following a traumatic event and then examine muscle phenylalanine kinetics in response to a known potent anabolic stimulus; essential amino acid ingestion. We found that while muscle phenylalanine kinetics were altered by acute hypercortisolemia, ingested EAA were still able to stimulate muscle protein synthesis and acutely reverse negative net phenylalanine balance. The present study was the first to investigate the time course and progression of the interaction between acute hypercortisolemia and protein metabolism in response to a bolus oral ingestion of an essential amino acid solution.

These data may reflect the very early stages of the catabolic process. Indeed, the increased circulating and muscle intracellular phenylalanine concentrations noted during the post-absorptive period raises the possibility that acute exposure to elevated cortisol levels primes the system by raising energy expenditure, increasing amino acid turnover and increasing amino acid movement between pools [5]. The net loss of nitrogen from muscle may not actually occur unless hypercortisolemia is prolonged, combined with inactivity [4] and/or combined with other mediators of the injury/trauma response [2, 15].

Ingestion or infusion of essential amino acids provides a strong anabolic stimulus [9, 10, 16]. Consistent with these previous data, we observed a significant increase in mixed muscle FSR in both experimental conditions during the 4hr period following EAA ingestion. Using the net balance approach to estimate the relationship between protein synthesis and breakdown, it is clear that hypercortisolemia acutely and transiently altered muscle phenylalanine kinetics immediately following EAA ingestion.

The mechanisms resulting in the altered phenylalanine concentrations and kinetics during hypercortisolemia are not entirely clear. Phenylalanine is an essential amino acid and is not synthesized or metabolized in the body, consequently changes in the rate of appearance or disappearance of plasma phenylalanine in the plasma reflects protein synthesis and breakdown [17]. The fact there was an increase in arterial and venous phenylalanine concentration, yet only a trend towards an increase in muscle protein breakdown (R_a) during hypercortisolemia suggests that increased protein breakdown in non-muscle tissues may have contributed to the elevated plasma amino acid levels during the pre- and post-EAA drink periods. Certainly, the liver represents a large potential source of amino acids that could be rapidly released into the circulation given an appropriate signal. There are some data suggesting that the gut may provide a source of amino acids during periods of exercise-induced stress [18, 19]. Using a dog model with an exercise intervention, Williams et al [18] reported an increased release of leucine from the splanchnic bed, with the primary contributing site being the gut. Certainly, given the high protein turnover rate in the gut, even a slight imbalance between synthesis and breakdown could produce relatively large changes in plasma amino acid concentrations.

The infusion of cortisol to produce hypercortisolemia in healthy human subjects has broad ranging effects and ultimately influences a range of hormonal responses including the regulation of glucose metabolism. Consequently, it is possible that the altered phenylalanine kinetics observed during hypercortisolemia may have also been influenced by the concomitantly elevated plasma insulin levels both before and following ingestion of 15g EAA. The mechanisms via which insulin regulates protein metabolism are complex and different organ systems and tissues may be affected in a variable manner (for a detailed review see Wolfe and Volpi [20]). Consequently, there remains a degree of ambiguity regarding the role of insulin on the in-vivo regulation of muscle protein synthesis and breakdown [20-22].

In the present study, we found that hypercortisolemia increased plasma glucose concentrations despite an accompanying increase in insulin levels [3, 5, 6, 23]. This elevation in plasma glucose levels was most likely due to a reduction in the rate of glucose disposal and not an increase in production [5, 24, 25]. The suggestion that there

was no increased requirement for glucose is congruent with the theory that acute exposure to hypercortisolemia primes the muscle for the subsequent use of glucose and amino acids for energy production if an additional stimulus presents (i.e., prolonged hypercortisolemia, inactivity, physical trauma).

In conclusion, the pattern of the anabolic response to amino acid ingestion is altered by hypercortisolemia. Nevertheless, dietary amino acids are still able to effectively stimulate muscle protein synthesis and reverse the negative net phenylalanine balance associated with hypercortisolemia and may provide an effective means of minimizing muscle loss during the first few days following debilitating injury.

Relations to Critical Path Roadmap. These findings have demonstrated that a defined nutritional intervention may be successful in countering the stress response associated with space flight. Further, this projects has provided the basis for testing of an operational countermeasure during actual space flight.

References:

1. Gore, D.C., et al., *Acute response of human muscle protein to catabolic hormones.* Ann Surg, 1993. **218**(5): p. 679-84.
2. Woolf, P.D., *Hormonal responses to trauma.* Crit Care Med, 1992. **20**(2): p. 216-26.
3. Darmaun, D., D.E. Matthews, and D.M. Bier, *Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production.* Am J Physiol (Endocrinol Metab), 1988. **255**(18): p. E366-E373.
4. Ferrando, A.A., et al., *Inactivity amplifies the catabolic response of skeletal muscle to cortisol.* J Clin Endocrinol Metab, 1999. **84**(10): p. 3515-3521.

5. Brillon, D.J., et al., *Effect of cortisol on energy expenditure and amino acid metabolism in humans.* Am J Physiol (Endocrinol Metab), 1995. **268**(31): p. E501-E513.

6. Simmons, P.S., et al., *Increased proteolysis: an effect of increases in plasma cortisol within the physiologic range.* J Clin Invest, 1984. **73**(February): p. 412-420.

7. Hart, D.W., et al., *Persistence of muscle catabolism after severe burn.* Surgery, 2000. **128**: p. 312-319.

8. Vary, T.C., *Regulation of skeletal muscle protein turnover during sepsis.* Curr Opin Clin Nutr Metab Care, 1998. **1**(2): p. 217-24.

9. Volpi, E., et al., *Exogenous amino acids stimulate net muscle protein synthesis in the elderly.* J Clin Invest, 1998. **101**: p. 2000-2007.

10. Volpi, E., et al., *Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction.* Am J Physiol Endocrinol Metab, 1999. **277**(40): p. E513-E520.

11. Wolfe, R.R., *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. 1992, New York: Wiley-Liss. 417-419.

12. Biolo, G., et al., *A new model to determine in vivo the relationship between amino acid transmembrane transport and protein kinetics in muscle.* JPEN, 1992. **16**: p. 305-315.

13. Jorfeld, L. and J. Warhen, *Leg blood flow during exercise in man.* Clin Sci, 1971. **41**: p. 459-473.

14. Bergstrom, J., *Percutaneous needle biopsy of skeletal muscle in physiological and clinical research.* Scand J Clin Lab Invest, 1975. **35**(7): p. 609-16.

15. Bessey, P.Q., et al., *Posttraumatic skeletal muscle proteolysis: the role of the hormonal environment.* World J Surg, 1989. **13**(4): p. 465-70; discussion 471.

16. Wolfe, R.R. and S. Miller, *Amino acid availability controls muscle protein metabolism.* Diab Nutri Metab, 1999. **5**(12): p. 322-328.

17. Wolfe, R.R., *Protein synthesis and breakdown.*, in *Radioactive and Stable Isotope Tracers in Biomedicine:Principles and Practice of Kinetic Analysis.* 1992, Wiley-Liss: New York. p. 377-417.

18. Williams, B.D., et al., *Gut proteolysis contributes essential amino acids during exercise.* Am J Physiol, 1996. **270**(1 Pt 1): p. E85-90.

19. Hourani, H., et al., *Effect of insulin-induced hypoglycemia on protein metabolism in vivo.* Am J Physiol, 1990. **259**(3 Pt 1): p. E342-50.

20. Wolfe, R.R. and E. Volpi, *Insulin and protein metabolism*, in *Handbook of Physiology*, A. Cherrington, Editor. 2001, Oxford: New York. p. 735-757.

21. Balon, T.W., et al., *Effect of insulin on protein synthesis and degradation in skeletal muscle after exercise.* Am J Physiol, 1990. **258**(1 Pt 1): p. E92-7.

22. Fryburg, D.A., et al., *Insulin and insulin-like growth factor-I enhance human skeletal muscle protein anabolism during hyperaminoacidemia by different mechanisms.* J Clin Invest, 1995. **96**: p. 1722-1729.

23. Gelfand, R.A., et al., *Role of counterregulatory hormones in the catabolic response to stress.* J Clin Invest, 1984. **74**: p. 2238-2248.

24. Lecavalier, L., G. Bolli, and J. Gerich, *Glucagon-cortisol interactions on glucose turnover and lactate gluconeogenesis in normal humans.* Am J Physiol, 1990. **258**(4 Pt 1): p. E569-75.

25. Shamoon, H., V. Soman, and R.S. Sherwin, *The influence of acute physiological increments of cortisol on fuel metabolism and insulin binding to monocytes in normal humans.* J Clin Endocrinol Metab, 1980. **50**(3): p. 495-501.

Presentations:

1. Alterations in Protein Metabolism During Space Flight and Inactivity (invited). 5th International Head-Out Water Immersion Symposium, Houston, TX, 9 October, 2002.

2. Hypercortisolemia alters muscle protein anabolism following ingestion of essential amino acids. 2002 Australian Conference of Science and Medicine in Sport. Melbourne. October 2002. D. Paddon-Jones, M. Sheffield-Moore, A.Sanford, S. Wolf, R. Wolfe & A. Ferrando.

3. Nutritional Interventions to Improve Muscle Protein Metabolism. Bioastronautics Investigators Workshop, Galveston, TX January 15th, 2003.

4. Protein Turnover During Spaceflight. Bioastronautics Investigators Workshop, Galveston, TX January 15th, 2003.

Abstracts:

ABSTRACT: Basal amino acid kinetics in human skeletal muscle during hypercortisolemia

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American College of Sports Medicine 2003

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Experimental Biology 2003

Articles:

Hypercortisolemia alters muscle protein anabolism following ingestion of essential amino acids. Douglas Paddon-Jones , Melinda Sheffield-Moore, Daniel L. Creson, Arthur P. Sanford, Steven E. Wolf, Robert R. Wolfe, and Arny A. Ferrando. American Journal of Physiology (Endocrinology and Metabolism) (In Press).

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